



Review article

Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer



Luka Andrisic^{a,b}, Danuta Dudzik^a, Coral Barbas^a, Lidija Milkovic^b, Tilman Grune^c,
Neven Zarkovic^{b,*}

^a CEMBIO (Centre for Metabolomics and Bioanalysis); Facultad de Farmacia; Universidad San Pablo CEU, Campus Montepríncipe, Madrid, Spain

^b Rudjer Boskovic Institute, Division of Molecular Medicine, Laboratory for Oxidative Stress, Zagreb, Croatia

^c German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany

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ABSTRACT

Association of oxidative stress with carcinogenesis is well known, but not understood well, as is pathophysiology of oxidative stress generated during different types of anti-cancer treatments. Moreover, recent findings indicate that cancer associated lipid peroxidation might eventually help defending adjacent nonmalignant cells from cancer invasion. Therefore, untargeted metabolomics studies designed for advanced translational and clinical studies are needed to understand the existing paradoxes in oncology, including those related to controversial usage of antioxidants aiming to prevent or treat cancer. In this short review we have tried to put emphasis on the importance of pathophysiology of oxidative stress and lipid peroxidation in cancer development in relation to metabolic adaptation of particular types of cancer allowing us to conclude that adaptation to oxidative stress is one of the main driving forces of cancer pathophysiology. With the help of metabolomics many novel findings are being achieved thus encouraging further scientific breakthroughs. Combined with targeted qualitative and quantitative methods, especially immunochemistry, further research might reveal bio-signatures of individual patients and respective malignant diseases, leading to individualized treatment approach, according to the concepts of modern integrative medicine.

1. Introduction

More than hundred types of malignant neoplasms affect humans and are commonly denoted as cancer. Although very different in place of origin and etiology they all share several common traits recognized today as the hallmarks of cancer [1]. These have been relatively well understood, suggesting pathophysiological association of oxidative stress with carcinogenesis [2]. Yet, even today, cancer patients largely depend on unspecific and often insufficient treatments, chemotherapy and radiation, utilized decades ago, which are often generating oxidative stress, too [3]. This paradox is further stressed by findings of carcinogenic as well as of selectively cytotoxic anti-cancer effects of products of lipid peroxidation, in particular of 4-hydroxynonenal (HNE) [4]. These, mostly recent, important findings request advanced

translational and clinical studies to understand the existing paradoxes in oncology, especially those related to the controversial use of antioxidants tending to prevent or even to treat cancer interfering with the complex mechanisms of the endogenous antioxidant mechanisms, which different between nonmalignant and cancer cells [2,5].

Aiming to improve biomedical understanding of systemic metabolic changes caused by carcinogenesis and by anti-cancer treatments metabolomics uses powerful tools in cancer research, diagnosis and therapy. It goes hand in hand with other –omics, together giving a more comprehensive picture. The ultimate goal of cancer research is finding reliable and specific biomarkers for early detection of cancer cells giving better survival prognosis for patients, along with finding specific metabolic targets for cancer therapies or providing insights into the mechanism of action of drugs used for anti-cancer treatments. Although

Abbreviations: HNE, 4-hydroxynonenal; NMR, nuclear magnetic resonance; MS, mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; MALDI-IMS, matrix-assisted laser desorption ionization mass spectrometry; SIMS, secondary-ion mass spectrometry; DESI, desorption electrospray ionization; NIMS, nanostructure-initiator mass spectrometry; HMDB, Human Metabolome Database; KEGG, Kyoto Encyclopedia of Genes and Genomes; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; GSH, glutathione; PPP, pentose phosphate pathway; Cys, cysteine; Gly, glycine; Glu, glutamate; GPx, glutathione peroxidase; GSSG, glutathione disulfide; CSSG, cysteine-glutathione disulfide; FH, fumarate hydratase; G6P, glucose 6-phosphate; OPA, ophthalmic acid; 2AB, 2-aminobutyric acid; MOC, metastatic tumors originating from primary ovarian cancer; EOC, primary epithelial ovarian cancer; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; LC, liver cirrhosis; GCS, γ -glutamyl-cysteine-synthetase; GS, glutathione synthetase; ESCC, esophageal squamous cell carcinoma; Kyn, Kynurenine

* Corresponding author.

E-mail address: zarkovic@irb.hr (N. Zarkovic).

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metabolomics already gave numerous valuable information about carcinogenesis, its applications in cancer research and clinical practice still remain elusive.

In metabolomics research many challenges exist tending to support modern concepts of personalized and integrative medicine. One of the major challenges is the sheer number of metabolites to be reckoned with, which is in particular difficult in case of advanced cancer due to its overall heterogenic nature [6]. Namely, although it is generally assumed that each tumor originates from a single malignantly transformed cell, with each cell division tumor cells progress forming neoplastic mass of mutually heterogenic cells with increasingly higher mutagenic potential. Ultimately, they give birth to cancerous cells with devastating metastatic phenotype and complex metabolic changes that might differ among malignant cells of the same tumor [1]. Further obstacles are found in xenometabolite interferences, sample collection, sample preparation, specificity and sensitivity of the current machines and methods utilized, while also tackling incomplete databases and system networks for easy identification of metabolites and metabolic pathways.

2. Metabolomics approach

2.1. The power of metabolomics

The 'omics' technologies (genomics, transcriptomics, proteomics, and metabolomics) have already impacted considerably the life sciences and yield important insights to advance our understanding of the pathophysiology of common complex diseases like e.g., diabetes [7,8], cardiovascular disease [9], asthma [10], Alzheimer [11] and cancer [12], among others. Each of the 'omics' is important for studies of complex biological systems and pathophysiological processes, complementing each other and the findings provided by the other, more targeted methods, especially by immunochemistry. However, metabolomics, as a downstream endpoint of all biological processes represents a core tool for a global assessment of metabolites within a biological system and reveals a specific 'snapshot' of the metabolic fingerprints under particular cellular processes that is closely related to the phenotype [13]. The metabolome consists of a complex mixture of thousands of metabolites, small-molecular-weight intermediates (typically with the molecular mass of 80–1500 Da), from a variety of metabolite classes including sugars, amino acids, oligopeptides, lipids, organic acids etc. The wide chemical diversity is further complicated by interferences of endogenous metabolome with the exogenous, i.e. with chemicals originating from environmental contaminants, drugs, additives, toxins and other xenobiotics [14–16]. Moreover, the size of the human metabolome is still being under estimation and might even exceed 100.000 molecules or more if we consider all intermediates and secondary metabolites [15]. In this countenance metabolomics is investigating complex biochemical interactions among different metabolites (metabolome) but is also building a global network to provide unique insight into metabolic reactions underlying activities of the proteins/peptides and gene expression in respect to the other bioactive molecules, including those important for the onset and control of oxidative stress and lipid peroxidation, as is the glutathione (GSH) system (Fig. 1).

3. State-of-the-art technologies in metabolomics

The development of robust, high-throughput metabolomic platforms, predominantly based on nuclear magnetic resonance (NMR) and mass spectrometry (MS) greatly facilitate metabolomics to address clinical questions as therapy and diet selection, treatment efficiency estimation, monitoring and eventually discovering novel biomarkers [16]. Due to the high complexity, compositional diversity of physico-chemical properties and concentration magnitude, one of the major analytical challenges in metabolomics is to generate a representative

coverage of the studied metabolome. Particularly the untargeted MS based multiplatform approaches, characterized by high sensitivity and reproducibility such as direct infusion MS (DIMS), liquid chromatography (LC-MS), gas chromatography (GC-MS) or capillary electrophoresis (CE-MS) significantly advances evaluating a complete set of metabolites [16,17]. The LC-MS is capable of detecting the widest range of metabolites, both small and large, polar and non-polar molecules, so the resulting data contain thousands of 'metabolic features', where each represents a unique mass-to-charge ratio in a given retention time value. Relatively easy sample preparation is undoubtedly an advantage, too. On the other hand, GC-MS with its strengths of high reproducibility, separation and easy metabolite identification pave the way for detection of small, volatile compounds (e.g., sugars, free fatty acids, organic acids, amino acids or Krebs cycle metabolites), although the technique has its limitations as sample derivatization is required. Additionally, CE-MS platform associated with high resolution offers a complementary approach for analysing polar or ionic compounds in complex aqueous matrices. Apparently, a limitation of LC-MS, GC-MS and CE-MS methods is the loss of spatial information that results upon metabolite extraction from the samples. Therefore, advanced molecular imaging approaches of metabolomics, such as MS-based matrix-assisted laser desorption ionization (MALDI-IMS) [18], secondary ion MS (SIMS) [19], desorption electrospray ionization (DESI) [20] or nanostructure-initiator mass spectrometry (NIMS) [21] can be an important alternative that provides positional information on the distribution of endogenous metabolites as well as for detection of administrated pharmaceuticals within tissues, thus offering a powerful tool to explore and monitor the effects of e.g. cancer metabolic reprogramming.

4. Metabolomics workflow

All metabolomics studies follow a common methodological pipeline from experimental design, sample collection and metabolite extraction, through data collection and analysis to biological interpretation [16,22]. To ensure a highest quality level of thus generated data, exceptional caution should be taken in each step of the workflow. In case of cancer related studies, where apart of blood plasma/serum or urine samples it is common to analyse tumor tissue, particular attention should be given to the sample harvesting and metabolite extraction. Tissues are complex structures composed of heterogeneous mixtures of morphologically and functionally distinct cell types, which is in case of cancer further complicated by the differences in cancer cell viability and inflammatory response to cancer growth and decay. Therefore, collection of representative and homogenous tissue sample requires critical evaluation which is challenging in general, but even more difficult in case of cancer samples which might comprise well-oxygenated regions around the growing rim, whereas the other, central regions might be necrotic [23]. For the tissue analysis it is also important to remove residual blood before storage to avoid the contamination coming from blood metabolome. Additionally, the ongoing biochemical reactions that could modify the metabolic content and provoke ex vivo alteration of sample composition should be stopped as soon as possible following sample harvesting (metabolism quenching) [23]. Such factors among many others may result in increased biological variability, which should be taken into account during data treatment and interpretation of thus obtained results.

5. Oxygen metabolism and redox balance

Aerobic organisms have evolved towards oxygen consumption to gain more efficient production of energy. In higher organisms oxygen is also involved in immunological responses, detoxification of xenobiotics, inflammation and neurotransmitter catabolism [24]. One of the consequences of oxygen metabolism is also production of reactive oxygen species (ROS), which are short-lived and very reactive, so they can react with all biological molecules, changing their structure and function.

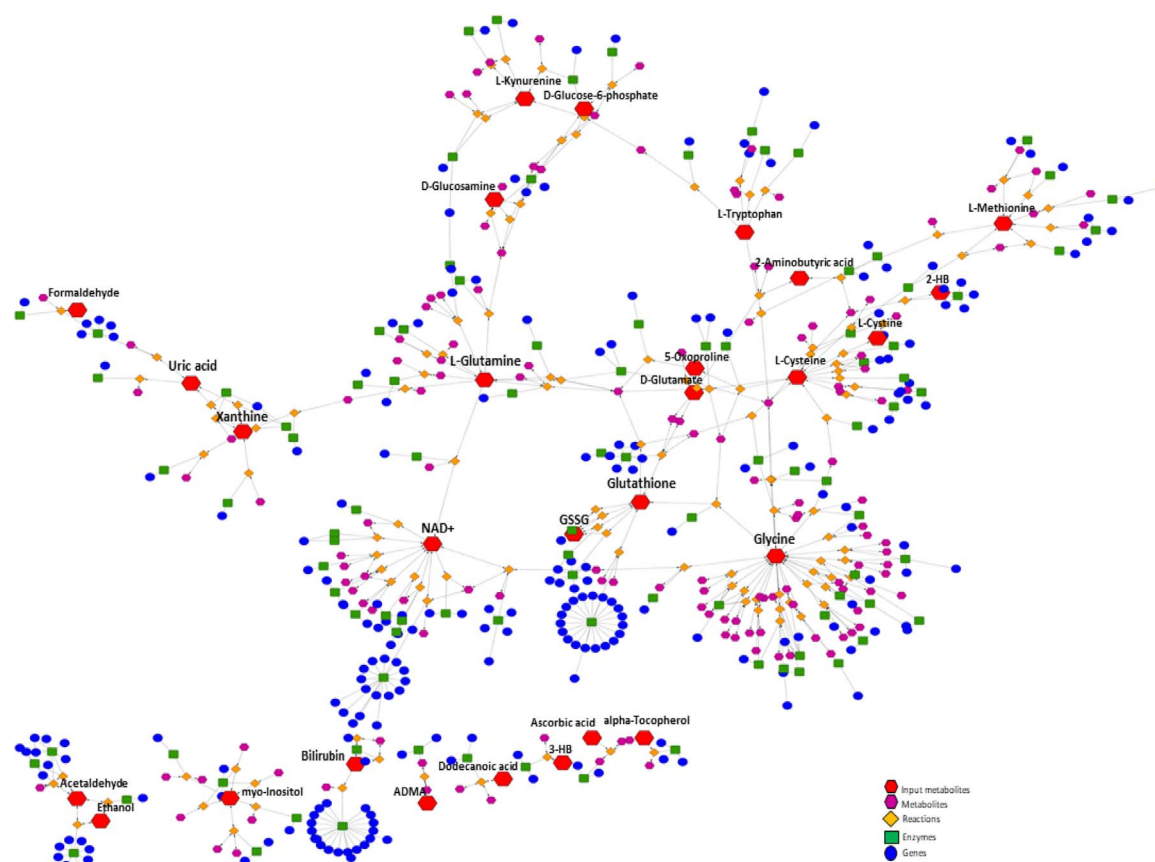


Fig. 1. Global biological network displaying complex interactions of altered metabolites (colored in red) reported to be connected with the redox signalling and cancer with other specific pathway-associated metabolites (fuchsia), enzymatic reactions (orange), enzymes (green) and related genes (blue). The study of the metabolome can be applied independently, or for global aspect in combination with other functional levels such as genomics, transcriptomics or proteomics systems. Genomics gives an overview of the genetic information provided by the DNA, transcriptomics looks into gene expression patterns, proteomics studies dynamic protein products and their interactions, while metabolomics is an intermediate step in understanding organism's entire metabolism and represents the closest link to phenotype. Image created by the Cytoscape ver. 3.5.1 platform for visualizing molecular interaction networks and biological pathways [112].

Under physiological conditions, ROS are constantly produced and removed within the cells. Although generally considered as harmful by-products of oxygen metabolism, ROS also have important physiological functions in signal transduction and immunological response [24]. A complex interplay between ROS generation, ROS signalling and ROS-induced damage are often species- and tissue-dependent being kept under control of elaborate enzymatic and non-enzymatic antioxidant defence systems [25]. However, overproduction of ROS or deregulation of antioxidant systems can lead to localized or systemic oxidative stress [26]. In its acute form moderate oxidative stress can boost cellular antioxidant capacities with positive, hormetic effects [27]. However, more severe, and especially chronic oxidative stress can lead to many deleterious effects on molecular level generating oxidized forms of proteins, lipids and DNA [28]. Harmful effects of chronic oxidative stress consider faster aging, DNA mutations, epigenetic changes that in worst cases cumulate with development and progression of many diseases including malignant alterations and cellular death [29]. Many processes at the cellular level depend on the potency to regenerate and maintain the optimal reducing power of the cell, which is mirrored through cellular redox state/balance [30]. When cells have weakened their redox buffering potential or when it is being compromised by overproduction of ROS, cellular redox state shifts more towards oxidation and the cell is likely to be susceptible to oxidative damage. Non-enzymatic antioxidants are usually consumed and enzymatic induced under such circumstances, including utilization of major cellular antioxidant, glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) [31]. Working in tandem with endogenous antioxidants, cellular redox balance is also dependent on essential nutrients

that are taken by food sources such as vitamins C and E, tannins, carotenes, dietary polyphenols and bioflavonoids, largely originating from vegetable food sources [32]. Therefore, success of any organism to defend against harmful oxidative stress largely depends on combination of inherited genetic as well as on the environmental factors. Type of food we eat, smoking habits and alcohol consumption as well as physical exercise, chemical agents and radiation give bases towards occurrence of oxidative stress and the readiness of an organism to defend against it [33].

6. Pathophysiology of oxidative stress

There are complex relations between oxidative stress and many pathophysiological conditions that might have beneficial consequences, as is inflammatory response to pathogens, but could also be harmful, initiating or enhancing degenerative, metabolic and auto-immune diseases or cancer development [34]. Hence, pathophysiology of oxidative stress should not be viewed as a hit or miss kind of event, but more as a cumulatively progressive process that gives fertile ground for development and progression of different, mostly stress and age associated diseases [29,35]. In a causally-consequential scheme of pathophysiology oxidative stress can be viewed as a cause and as a consequence of various diseases, giving significance to the idea of personalized and integrative medicine [36]. That is in a large part dependent on the assumption that harmful long-term effects of oxidative stress can still be prevented, treated and even reversed if monitored well and in time [37]. One of the goals of the preventive medicine approach should be a definition of the health state of an organism through a personalized

medicine approach and systematic monitoring of oxidative stress occurrence from young age throughout one's lifespan. The challenges of such an oxidative stress research lies in finding reliable and specific biomarkers of oxidative stress in respect to specific pathophysiological conditions and diseases [38,39]. Direct biomarkers of oxidative stress, ROS, especially free radicals are short-lived, labile and, accordingly, hard to measure. Therefore, more stable, secondary products/metabolites of oxidative stress are mainly utilized in scientific research. They include oxidized forms of proteins, lipids and DNA as well as their degradation products [40,41]. A complementary way of assessing onset of oxidative stress is by determination of antioxidants and their related metabolites [42]. So far, biomarkers of oxidative stress were considered to be too variable and highly unspecific for utilization in medical diagnostics of a specific disease. In scientific and clinical research they were mostly utilized to assess involvement of oxidative stress in a certain type of disease, but were usually considered too much unspecific to give specific clues regarding diagnosis or therapy [38]. With development of metabolomics, a new window of possibilities is opening, where many more biomarkers of oxidative stress could be assessed in one sample. At the same time metabolomics could generate more complex pathophysiological and metabolic schemes but could also give greater potential for solving puzzles that go beyond generalization and more towards specific and personalized approach in fighting human diseases. To do so, metabolomics should be combined with targeted, specific analysis like immunochemistry.

7. Oxidative stress and cancer metabolic switch

Malignant alterations at genetic level are the main pathological driving force of carcinogenesis, often being associated with oxidative stress [43]. ROS are the most important naturally occurring mutagenic factors in the organism that cause genetic instability within the cells. Multiple biochemical reactions where oxygen is metabolized can lead to generation of reactive toxic intermediates that may cause DNA damage [44]. From evolutionary point of view, at a normal rate, harmless mutagenesis gives an advantage for the cell or organism to adapt to new conditions via mutagenic changes of DNA. Chronic oxidative stress mimics this naturally occurring process but on a much larger scale, giving more rigorous pressure on the cells and ultimately forcing cells to adapt to these new conditions or end in cell death [28,45]. Many adaptive changes, cumulatively increasing over longer periods of time, need to occur within one cell for malignant transformation to take place. There are many examples how genetic mutations (inherited or acquired) lead to increased ROS production, which causes DNA damage and contributes to malignant transformation [45,46]. Therefore, cancer cells in a way depend on this highly instable and mutagenic environment of oxidative stress.

Beside general characteristics, described as hallmarks of cancer, these highly resistant transformed cells are characterized by different metabolic patterns supporting sustained proliferation of cancer cells and higher resistance to oxidative stress [45,47]. Generally speaking, metabolism of developed tumors is different from that of normal cells. However, large diversity also exists between different tumor types. Depending on their tissue origin tumor cells retain a heterogeneous expression of tissue specific genes and a metabolic networks resembling that of the original tissue that tumor arose from [47,48]. Cumulatively, metabolic phenotype of every tumor is a complex interplay of genetic mutations favoring tumorigenesis, specific metabolic prints of tissue of tumor origin and metabolic “cross talk” between tumor and its micro-environment and nutrient uptake [48–50]. Symbiotic metabolic relationship also exists between cancer cells and supportive, non-malignant stromal cells that form jointly heterogeneous tumor mass [51]. In addition, only a small part of cells with mutated genome exhibit metabolic phenotypes that support continuous proliferation, while all of them have to adapt their metabolic requirements to survive within harsh condition of the surrounding wildly growing malignant cells [47].

All these diverse metabolic networks are still far from reach of understanding, at least in a way that would be useful in exploiting cancer dependent metabolic alterations in new therapeutic approaches.

Oxidative stress is involved in all aspects of tumor development and progression, but often also in anti-cancer therapies [45]. Strong impact of oxidative stress on tumor development can be viewed at the sites of chronic inflammation that causes oxidative stress mostly through complex reactions of cellular immune system, but also through cytokine bioactivities [28]. Increased production of ROS is observed upon activation of many different oncogenes or mutations of tumor suppressor genes further influencing additional DNA mutations [45]. Increased ROS production is also involved in induction of angiogenesis, epithelial-mesenchymal transition (EMT) and “cross-talking” with surrounding cells that in return support tumorigenesis together driving forward tumor development [45]. Thus, oxidative stress has pivotal role in metabolic switch that drives cells towards adaptation to carcinogenic stressors and malignant transformation. Maybe the most striking metabolic feature of many tumors is strong dependence on anaerobic glycolysis despite functional mitochondria and oxygen availability [52]. Explanation behind this switch may lie in the fact that despite being energetically less efficient process, glycolysis generates ATP at a much faster rate or by avoiding mitochondrial oxidative phosphorylation, cancer cells are also protected from additional source of ROS that could prove to be deleterious in these highly proliferating and altered metabolizing conditions already burdened by enhanced ROS production [53–55]. In addition, it is viewed by many that importance of glycolysis in this matter is to support high proliferating cancer cells with substrates of pentose phosphate pathway (PPP) for nucleotide synthesis as well as for the NADPH production, further supporting cellular redox balance and protection against oxidative stress [56,57]. Intensive glycolysis of cancer cells is accompanied by increased production of lactate that is exported in surrounding tissue causing highly acidic microenvironment, which can be further supported by inflammation. Local acidosis drives tumor cells to adapt and develop phenotype resistant to acid-induced cell toxicity giving them strong advantage towards more proliferation and invasion [58]. Interestingly, not all tumor types show intense glycolysis. This metabolic phenotype is mostly observed in rapidly growing cancer cells. Some evidence suggests that metabolic utilization of both glycolysis and mitochondrial oxidative phosphorylation in tumor cells could be rapidly switched in favor of one or the other mainly depending on micro-environmental challenges as in the case of low glucose levels [59,60]. A high metabolic rate of cancer cells is accompanied by increased ROS production. However, this increment in ROS is not as damaging for tumor cells as it would be for normal counterpart cells, because tumor cells can induce new, cancer specific mechanisms of redox balance which is the backbone of their adaptive process supporting high proliferative rates while surviving intensive tumorigenic metabolic rates and harsh tumor micro-environment [45]. Such an increased resistance to oxidative stress could be one of the major advantages acquired by the transforming cells allowing them more efficient bypass of ROS damage while also giving them an important leverage compared to normal cells in the fighting chances for survival [45]. On the other hand, cancer invasion as well as inflammatory response to cancer can also induce lipid peroxidation in adjacent non-malignant tissue that can response by increased lipid peroxidation generating cytotoxic lipid peroxidation product HNE to defend themselves and the organism against invading cancer even by formation of the protein adducts of HNE [61,62]. Hence, it is not surprising that among common metabolic features of cancer cells is alteration of the biomembrane lipid profile by reducing PUFAs to prevent lipid peroxidation, since thus generated HNE can exert more potent cytotoxic, pro-necrotic and pro-apoptotic effects for cancer than for nonmalignant cells, also regulating activities of numerous genes, enzymes and cytokines involved in the growth regulation and oxidative homeostasis [63,64]. Therefore, metabolomic analysis targeting lipid metabolism and oxidative stress in cancer should be complemented by

quantitative and qualitative immunochemical analysis for the lipid peroxidation products, notably for HNE-protein adducts, well known already as important biomarker of oxidative stress in various diseases, including cancer [65,66].

8. Metabolomics insights into redox state of cancer

Most metabolomics studies featured in this review used an untargeted approach on platforms mostly utilized in metabolomics research; HPLC-MS, GC-MS, CE-MS or NMR while also using different biological samples; urine, plasma, serum, tissue and cultured cells. Relevant findings of altered metabolomic patterns in cancer reflecting oxidative stress have been summarized. It is important to mention that non-targeted analysis is not the best way to measure labile compounds that are very easily oxidized such as glutathione (GSH) and attention should be paid to experimental conditions when evaluating such results, because rapid changes of GSH are well known, mainly in blood, unless collected under especial conditions.

Glutathione plays a central role in cellular antioxidant defence system. Its participation in cellular metabolic redox processes and ROS scavenging is ubiquitous in aerobic organisms [67]. A relatively simple structure of this soluble tripeptide does not reveal its powerful versatile bioactivities especially its antioxidant properties. GSH is a non-enzymatic antioxidant synthesized intracellularly from cysteine (Cys), glycine (Gly) and glutamate (Glu) and is highly abundant in all major cell compartments like cytosol, nuclei and mitochondria. In the nucleus GSH protects sulphydryl groups of proteins that are essential for DNA repair and expression. Its major antioxidant properties are further manifested in direct scavenging of hydroxyl radicals and singlet oxygen, while it can also detoxify hydrogen peroxide (H_2O_2), lipid peroxides and even HNE in tandem with enzymatic action of glutathione peroxidase (GPx) and glutathione transferases. GSH is also involved in reductive regeneration of important antioxidants including water soluble vitamin C and lipid soluble vitamin E [67]. The oxidized form of GSH is glutathione disulfide (GSSG), formed by oxidation of two molecules of GSH. The GSSG is usually formed during reduction of organic hydroperoxides (ROOH) and inorganic peroxides like H_2O_2 in enzymatic reaction catalyzed by GPx or peroxiredoxins. In return, GSSG can be reduced back to GSH in tandem enzymatic action of glutathione reductase (GR) and the reducing equivalent NADPHH+. Therefore, the GSH:GSSG ratio is considered as an important indicator of redox balance in cells, with a higher ratio signifying less oxidative stress [68].

It is not surprising that GSH metabolism was found to be largely perturbed in different cancer types in many of the studies mentioned in this paper. The human liver tumor cell line, HepG2 exhibited significant reduction of GSH upon treatment with H_2O_2 , while the same cells pretreated with vitamin E and GSH showed increased GSH in comparison to control [69]. Another study on non-small-cell lung cancer cells (NSCLC) examined metabolic patterns between three isogenic cell clones harboring different KRAS mutations at most frequently mutated loci, codon-12. In general, KRAS mutations lead to continuous activation of pathways that increase proliferation and avoid apoptosis such as MAPK and PI3K/AKT7/mTOR pathways [70]. The intracellular redox state of the KRAS mutants, measured by GSH, GSSG and the ratio GSH/GSSG was comparable to the wild type. Despite that, KRAS mutants showed metabolic shift towards an oxidized state. In addition, small but significant differences of redox state could also be observed between different KRAS mutants [71]. Metabolic profiling of three different breast cancer cell lines (MDA-MB-231, –453 and BT-474) showed significant decreases in GSH as well as GSH/GSSG ratios compared to the control epithelial cell line MCF-10A [72]. In reference to these results the GSH/GSSG ratio was found to be significantly decreased in the blood of breast cancer patients when compared to control subjects [73]. A highly malignant form of renal cancer is associated with mutations in an enzyme of tricarboxylic cycle (TCA), the fumarate hydratase (FH) [74]. An interesting study based on a computational predictive model

followed by metabolomic analysis revealed induction of chronic oxidative stress in cells deficient in FH in a process of continuous GSH depletion [46]. In this in vitro mouse model of FH-deficient kidney cells (Fh1^{Δ/Δ}) fumarate was found to be aberrantly accumulated and consequently GSH was succinated in non-enzymatic reaction forming succinic-GSH. In this case GSH succination ultimately led to larger steady-state pool of GSH by provoking compensational biosynthesis of new GSH. However, despite this fact, cells were affected by disturbed redox balance in consequence to NADPHH+ depletion that was being exhausted for newly synthesized GSH [46]. Persistent oxidative stress elicited by succination of GSH induced cellular senescence in vitro and in vivo and provoked initiation of renal cancer in the mouse model when senescence was bypassed [46]. The MYCN gene is amplified and overexpressed in a large proportion of high stage neuroblastoma patients and has been recognized as a key driver of tumorigenesis. TH-MYCN transgenic mice expressing human MYCN gene develop murine equivalent of neuroblastoma with all the main signatures of human disease [75]. An interesting in vivo study that examined neuroblastoma of TH-MYCN mice in three different stages of tumor development (pre-tumor, early tumor and advanced tumor) revealed high dependence of carcinogenesis on the GSH metabolism and activities. The most striking difference in GSH production, compared to wild type, was observed in advanced tumors [76]. Complementary to that, the same study showed increased levels of endogenously produced amino acids, which comprise main building blocks for glutathione synthesis (Cys, Glu and Gly) and also glutamine that is readily converted to glucose when needed. Elevated levels of 5-oxoproline and cysteine-glutathione disulfide (CSSG) were also found [76]. The 5-oxoproline is uncommon naturally occurring amino acid derivative that is converted to glutamate by 5-oxoprolinase in the glutathione cycle [77], while CSSG is a molecule that is formed upon oxidative stress of glutathione [78]. Thus, both CSSG and GSSG can be considered as biomarkers of oxidative stress. Significant alterations in CSSG levels were specifically observed in advanced stage of cancer [76]. The CSSG levels were also found to be highly upregulated in cancer tissue in a mouse xenograft model of human kidney cancer [78]. Furthermore, in three different clinical studies that included cancer tissue analysis of head and neck squamous cell carcinoma (HNSCC), breast cancer (ER+/ER-) and oral cancer, metabolomics again revealed strong dependence of analyzed cancer cells on the increased GSH [79–81]. Investigating oral cancer tissue showed that beside increase in oxidized and reduced form of GSH, many metabolites involved in GSH biosynthetic pathway were also strongly upregulated (Table 1) [79]. In HNSCC, a high increase of GSH was also observed as well as increase in amino acids Gly, Gln and Glu further supporting GSH synthesis [80]. Both, estrogen-receptor positive cancers (ER+) and estrogen-receptor negative cancers (ER-) showed increased glutathione levels (GSH and GSSG) compared to adjacent healthy tissue. However, ER- cancer cells exhibited higher glutathione levels compared to ER+ cancers, suggesting increased GSH synthesis in order to counteract higher levels of oxidative stress in ER- cancers. Complementary to these findings CSSG levels were also found to be more increased in ER- patients [81]. Importance of the redox balance in malignant transformation was further corroborated in a clinical study comparing metabolic signatures in the progression of hepatocellular carcinogenesis from hepatitis B virus (HBV) and liver cirrhosis (LC) to hepatocellular carcinoma (HCC) [82]. A significant increase in all amino acids related to GSH synthesis including 5-oxoproline were observed in serums of HCC patients [82]. In addition, HCC patients showed a high increase of glucose 6-phosphate (G6P), the starting point of glycolysis but also PPP, important source of NADPHH+ for the generation of reduced GSH [82]. Similar results were obtained in another large clinical study where the metabolic profiling of tissue samples from patients with colorectal cancer was performed. Again, there was a significant increase in all GSH building amino acids including 5-oxoproline, revealing strong dependence of colorectal cancer cells to increased GSH consumption [83]. In addition, 2-aminobutyric acid

Table 1
Metabolites related to oxidative stress perturbed in cancer cells.

Altered metabolite (HMDB ID)	Analytical system	Type of study//studied sample	Significant change in tumor	Ref.
GSH (HMDB00125)	HPIC-MS	<i>In vitro</i> HepG2, human liver tumor cell	↓ (HEPG2+H ₂ O ₂)	[69]
	LC-MS/MS; LC-SRM-MS *	<i>In vitro</i> human non-small-cell lung cancer (NSCLC), three isogenic NSCLC cell clones overexpressing 3 mutated forms of KRAS at codon –12 (G12C, G12D, G12V)	↓ (significant only in G12D)	[71]
	LC-MS/MS	<i>In vitro</i> mouse kidney FH-deficient cell lines Fh1 ^{Δ/Δ} and controls Fh1 ^{fl/fl}	↑	[46]
	UPLC-ESI-Q-TOF	<i>In vitro</i> 3 breast cancer cell lines vs control epithelial breast cells	↓	[72]
	UPLC-MS/MS (ESI +/-) mode, GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
GSSG (HMDB03337)	HR-MAS NMR	Clinical study / head and neck squamous cell carcinoma (HNSCC), matched normal adjacent tissue (NAT), tumor and lymph-node metastasis (LN-Met) //tissue	↑ (tumors and metastasis)	[80]
	GC-MS, LC-MS	Clinical study / Breast cancer/ER+ /ER-/- associations between genetics and metabolism// tissues	↑↑ ER-↑ ER+	[81]
	CE-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑ (tissue)	[79]
	LC-MS/MS; LC-SRM-MS *	<i>In vitro</i> human non-small-cell lung cancer (NSCLC), three isogenic NSCLC cell clones overexpressing 3 mutated forms of KRAS at codon –12 (G12C, G12D, G12V)	↑ trend, not significant	[71]
	UPLC-ESI-Q-TOF	<i>In vitro</i> 3 breast cancer cell lines vs control epithelial breast cells	↑ (in two breast cancer cell lines)	[72]
Glycin (HMDB00123) e	GC-MS, LC-MS	Clinical study / Breast cancer/ER+ /ER-/- associations between genetics and metabolism// tissues	↑↑ER-↑ ER+	[81]
	CE-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑(tissue)	[79]
	UPLC-MS/MS (ESI +/-mode), GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
	GC-MS-TOF	Clinical/ Hepatocellular carcinoma (HCC) //serum	↑	[82]
	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↑	[83]
Cysteine (HMDB00574)	GC-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑(in tissue)	[79]
	UPLC-MS/MS (ESI +/-mode), GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
	GC-MS	Clinical/ Lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- Non metastasis vs metastasis vs controls //serum	↓	[93]
	GC-MS-TOF	Clinical/Adenocarcinoma type of non-small-cell lung cancer (NSCLC)// tissue	↑	[94]
	GC-MS-TOF	Clinical/ Hepatocellular carcinoma (HCC) //serum	↑	[82]
Glutamate (HMDB03359)	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↑	[83]
	UPLC-MS/MS (ESI +/-mode), GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
	HR-MAS NMR	Clinical study / head and neck squamous cell carcinoma (HNSCC), matched normal adjacent tissue (NAT), tumor and lymph-node metastasis (LN-Met) //tissue	↑ (tumors and metastasis)	[80]
	GC-MS-TOF	Clinical/ Hepatocellular carcinoma (HCC) //serum	↑	[82]
	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↑	[83]
Glutamine (HMDB00641)	CE-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑(tissue)	[79]
	UPLC-MS/MS (ESI +/-mode), GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
	HR-MAS NMR	Clinical study / head and neck squamous cell carcinoma (HNSCC), matched normal adjacent tissue (NAT), tumor and lymph-node metastasis (LN-Met) //tissue	↑ (tumors and metastasis)	[80]
	E-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑(tissue)	[79]
	GC-MS and LC-MS/MS	Clinical / normal ovarian tissue vs. primary epithelial ovarian cancer (EOC) vs. metastatic tumors resulting from primary ovarian cancer (MOC)// tissue	↑MOC	[86]
5-oxoproline (HMDB00267)	UPLC-MS/MS (ESI +/-mode), GC-MS	Animal / mouse model of neuroblastoma// neural tissue or tumors	↑ (from early to advanced tumor)	[76]
	GC-MS-TOF	Clinical/ Hepatocellular carcinoma (HCC) //serum	↑	[82]
	CE-MS-TOF	Clinical study / oral cancer, tumor and matched control // tissues and unstimulated saliva	↑(tissue)	[79]
	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↑	[83]
	LC-MS/MS; LC-SRM-MS *	<i>In vitro</i> human non-small-cell lung cancer (NSCLC), three isogenic NSCLC cell clones overexpressing 3 mutated forms of KRAS at codon –12 (G12C, G12D, G12V)	↓	[71]
Cystine (HMDB00192)	LC-MS	Clinical/epithelial ovarian cancer (EOC) primary EOC vs post-operative EOC patients vs recurrent EOC//plasma samples	↓ (post operative/preoperative)/(relapsed/postoperative)	[95]
	GC-MS	Clinical/ lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- Non	↑	[93]

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Table 1 (continued)

Altered metabolite (HMDB ID)	Analytical system	Type of study//studied sample	Significant change in tumor	Ref.
	GC-MS	Clinical/ Lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- non metastasis vs metastasis //serum	↓Metastasis ↓Nonmetastasis	[93]
	GC-MS-TOF	Clinical/glioblastoma, screen of prediagnostic samples collected 0.5–22 years before glioblastoma diagnosis. //serum	↑(in prediagnosed serum)	[89]
α -tocopherol, 8-tocopherol, γ -tocopherol	GC-TOF	Clinical/Adenocarcinoma a type of non-small-cell lung cancer (NSCLC) // tissue	↑	[94]
	GC-MS and LC-MS/MS	Clinical / normal ovarian tissue vs. primary epithelial ovarian cancer (EOC) vs. metastatic tumors resulting from primary ovarian cancer (MOO)//tissue	↑↑MOC (α, δ)	[86]
Uric acid (HMDB00289)	LC-MS	Clinical/epithelial ovarian cancer (EOC) primary EOC vs post-operative EOC patients vs recurrent EOC//plasma samples	↑ (post operative)↓(relapsed)	[95]
	GC-TOF	Clinical/Adenocarcinoma a type of non-small-cell lung cancer (NSCLC) // tissue	↑	[94]
Kynurenine (HMDB00684)	CE-MS-TOF	Clinical study / oral cancer; tumor and matched control // tissues and unstimulated saliva	↑ (tissue)	[79]
	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↑	[83]
	GC-MS, LC-MS	Clinical study / Breast cancer/ER+ /ER- associations between genetics and metabolism// tissues	↑ER-↑↑ER+	[81]
	GC-MS and LC-MS/MS	Clinical / normal ovarian tissue vs. primary epithelial ovarian cancer (EOC) vs. metastatic tumors resulting from primary ovarian cancer (MOO)//tissue	↑ EOC	[86]
Myo-inositol (HMDB00211)	GC-MS-TOF	Clinical/ colorectal cancer in relation to matched non tumor// tissues	↓	[83]
	GC-MS-TOF	Clinical/glioblastoma, screen of prediagnostic samples collected 0.5–22 years before glioblastoma diagnosis. //serum	↑(in prediagnosed serum)	[89]
Xanthine (HMDB00292)	GC/MS, UPLC/MS-MS	Clinical/ Hepatocellular carcinoma (HCC) arising from hepatitis C (HCV); HCC, hepatitis C cirrhosis, disease controls (DC) and healthy volunteers //serum	↑ (HCC vs DC)	[88]
	GC-TOF	Clinical/Adenocarcinoma a type of non-small-cell lung cancer (NSCLC) // tissue	↑	[94]
	NMTR	Animal /rat model of gastric carcinogenesis//serum	↑	[102]
	GC-MS and LC-MS/MS	Clinical / normal ovarian tissue vs. primary epithelial ovarian cancer (EOC) vs. metastatic tumors resulting from primary ovarian cancer (MOO)//tissue	↑EOC	[86]
Lauric acid (HMDB00638)	GC-MS	Clinical/melanoma Pilot study (small cohort)- //biopsied tissue melanoma and matched control non-neoplastic skin from the same patient (VOC)	↑	[103]
Alkanes such as ethane and pentane	GC-MS	Review breath cancer/in vivo vs. in vitro hypoxic and hyperoxic cond// Volatile organic compounds (VOC)	↑	[104]
Aldehydes, alcohols and ketones	GC-MS	Review breath cancer/in vivo vs. in vitro hypoxic and hyperoxic cond// Volatile organic compounds (VOC)	↑	[104]
Indoxyl sulfate (HMDB00682)	LC-MS	Clinical/epithelial ovarian cancer (EOC) primary EOC vs post-operative EOC patients vs recurrent EOC//plasma samples	↑ (post operative)↓(relapsed)	[95]
Asymmetric dimethylarginine (HMDB01539)	H NMR	Clinical/Esophageal adenocarcinoma (EAC) Barrett's esophagus and EAC. // urine	↑ (EAC)	[85]
	UPLC-ESI-QTOF/MS	Animal /rodent squamous cell carcinoma (SCCVII) injected into the subcutaneous space of female C3H/He mice temporal study//. serum, liver, and tumor	↑	[105]
24-nor – 5 β -cholate-triol (NA)	UPLC-MS	Animal /Dicer-Pten double knockout (DKO) mice. Simulates High-grade serous carcinoma (HGSC) in women – early tumor (ET) // serum	↑	[106]
Bilirubin (HMDB00054)	UPLC-MS	Animal /Dicer-Pten double knockout (DKO) mice. Simulates High-grade serous carcinoma (HGSC) in women – early tumor (ET) // serum	↓	[106]
Methionine (HMDB00696)	GC-MS	Clinical/ Lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- non metastasis vs metastasis //serum	↓	[93]
Methylcysteine (HMDB02108)	GC/MS	Clinical/ Lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- Non metastasis vs metastasis //serum	↓	[93]
Pyrogallol (NA)	GC-MS	Clinical/ Lymph node metastasis of esophageal squamous cell carcinoma (ESCC)- non metastasis vs metastasis //serum	↓	[93]
Malic acid (HMDB00744)	HPLC/MS	In vitro/HepG2/ human liver tumor cell	↓HEPG2 + H ₂ O ₂	[69]
Methionine sulfoxide (HMDB02005)	CE MS and LC MS	Clinical/ nine types of liver disease and healthy controls// serum	↑	[87]
Glucosamine (HMDB01514)	CE MS and LC MS	Clinical/ nine types of liver disease and healthy controls// serum	↑	[87]
Indole – 3-propionic acid (HMDB02302)	rapid resolution LC MS	Clinical/Epithelial ovarian cancer (EOC) primary EOC vs post-operative EOC patients vs recurrent EOC//plasma samples	↓ (primary) ↑ (post operative)↓(relapsed)	[95]
Erythritol (HMDB02994)	GC-MS-TOF	Clinical/glioblastoma, screen of prediagnostic samples collected 0.5–22 years before glioblastoma diagnosis. //serum	↑(in prediagnosed serum)	[89]
erythronic acid (HMDB00613)	GC-MS-TOF	Clinical/glioblastoma, screen of prediagnostic samples collected 0.5–22 years before glioblastoma diagnosis. //serum	↑(in prediagnosed serum)	[89]

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Table 1 (continued)

Altered metabolite (HMDB ID)	Analytical system	Type of study//studied sample	Significant change in tumor	Ref.
Glucose 6-phosphate (HMDB01401)	GC-MS-TOF GC-MS and LC-MS/MS	Clinical/ Hepatocellular carcinoma (HCC) //serum Clinical / normal ovarian tissue vs. primary epithelial ovarian cancer (EOC) vs. metastatic tumors resulting from primary ovarian cancer (MOC) // tissue	↑ ↑MOC	[82] [86]

(2AB), a key intermediate in the biosynthesis of ophthalmic acid (OPA) was significantly elevated in cancer patients. Since OPA is a tripeptide analogue of glutathione in which the cysteine group is replaced by 2AB [84], OPA can be synthesized from 2AB and glutamate by the enzyme γ -glutamyl-cysteine-synthetase (GCS) to form c-glutamyl-2 aminobutyrate, which can be further catalyzed by glutathione synthetase (GS) to form OPA, which was previously reported to be potential biomarker for hepatic GSH depletion following oxidative stress [84] and was also found to be upregulated in breast cancer study mentioned previously, showing higher levels in ER- compared to ER+ cancers [81,84]. Complementary to that, high 2AB levels can also reflect increased oxidative stress [84]. Few other clinical studies considering metabolomics approach showed upregulation of 2AB in esophageal carcinoma and oral cancer [79,85]. Additionally, in a clinical study that explored metabolic patterns of primary epithelial ovarian cancer (EOC) and metastatic tumors originating from primary ovarian cancer (MOC) 2AB was elevated in both [86]. In this study, OPA was also found to be significantly upregulated but only in MOC patients. Another interesting biomarkers of cancer that can be connected to perturbed GSH metabolism are γ -glutamyl dipeptides. These were found to be upregulated in the serum of hepatocellular carcinoma patients [87,88]. In general, γ -glutamyl dipeptides are synthesized by ligation of glutamate with various amino acids and amines by the action of GCS being feedback-inhibited by GSH and the levels of γ -glutamyl dipeptides that are indicative of the amount of cellular GSH [87]. The 2-hydroxybutyric acid (2HB) can also be considered as one of the distinguishing features in relation to GSH metabolism. It is primarily produced in mammalian hepatic tissues, which catabolize threonine or synthesize glutathione. Under conditions of intense oxidative stress hepatic glutathione synthesis is increased and in high demand for cysteine. In such cases homocysteine is diverted from the transmethylation pathway transferring methionine by this pathway to cystathionine, which is further cleaved to cysteine and finally incorporated into glutathione. The 2HB is then released as a by-product of cystathionine conversion to cysteine, which was found to be increased in sera of patients suffering from hepatocellular carcinoma as well as in cancer tissue of EOC and MOC patients in the same study mentioned earlier [86,88].

Among the most important nutrients for a healthy redox balance in human organism are tocopherol (vitamin E) and ascorbic acid (vitamin C), which were also found to be perturbed in some cancers (Table 1). In the metabolomic study using sera samples of clinically silent glioblastoma cases were compared with matching sera of patients with the manifested glioblastoma to find that high levels of α -tocopherol and γ -tocopherol in the sera of patients with clinically silent tumors implying possible connection of increased blood tocopherol levels with initiation of glioblastoma [89]. However, it may be also possible that even in such cases tocopherol could still have its primarily have (ineffective) preventive role as antioxidant to terminate the onset of lipid peroxidation in glial cells, since lipid peroxidation is known to occur with intensity proportion to the level of malignancy of this particular brain tumors [90,91]. Namely, tocopherol is an essential micronutrient involved in various oxidative stress-related processes, especially protection of unsaturated fatty acids from oxidation [92]. Another clinical metabolomics study of esophageal squamous cell carcinoma (ESCC) that also included lymph node metastasis again found decreased levels of α -tocopherol and γ -tocopherol in the sera of patients with developed cancers [93]. On the other hand; a few metabolic analysis of cancer tissue samples detected increased levels of α - and γ -tocopherol [81,86,94], as in case of ER- breast cancer patients who had significantly increased tocopherol levels if compared to the patients with ER+ cancer [81], while tocopherol levels were in malignant tissue higher than in adjacent mammary gland tissue. Similarly, vitamin C was shown to be significantly increased in tissue of breast cancer patients, again with much higher levels measured in ER- cases. In an ovarian cancer study, metastatic ovarian cancer (MOC) cases showed striking increase in δ - and γ -tocopherol when compared with primary epithelial ovarian cancer

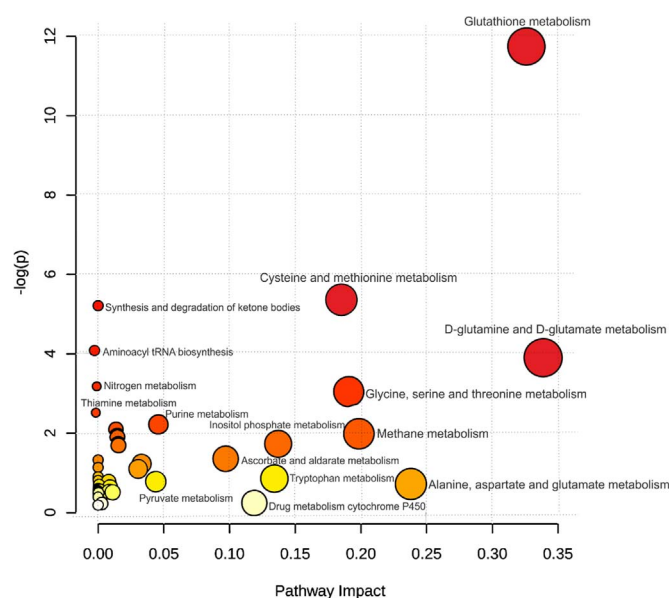


Fig. 2. Schematic representation of significantly altered metabolic pathways associated with the altered redox signalling in cancer. The metabolite ID matching with KEGG and HMDB was possible for 72 hits, the analysis was adjusted by a hypergeometric algorithm, and the impact on pathway topology was based on relative-betweenness centrality. Metabolic pathway analysis were performed using MetaboAnalyst 3.0 software [113].

(EOC) and control samples. Interestingly, EOC patients did not show any difference if compared to control [86].

In another study, the plasma samples of patients with primary EOC were compared with plasma obtained from surgically treated EOC patients and from those with recurrent EOC [95]. Biomarkers of oxidative stress like 3-indolepropionic acid and 3-indoxylsulfate were down-regulated in recurring EOC patients and downregulation of 3-indolepropionic acid was also observed in primary EOC patients. The 3-indolepropionic acid is a product of tryptophan deamination that has been previously found to have antioxidant properties protecting at least some cells, including neurons from ischemia-induced DNA damage and lipid peroxidation [95,96]. On the other hand, 3-indoxylsulfate has been shown to propagate oxidative stress by strongly decreasing circulating levels of glutathione [85,97]. In contrast to this result, urine levels of 3-indoxylsulfate were found to be upregulated in patients with esophageal carcinoma invoking the possible role of 3-indoxylsulfate induced oxidative damage in the malignant transformation of esophageal cancer [85]. Another reducing agent with strong antioxidant properties in blood, uric acid, was found to be downregulated in plasma samples of recurring EOC patients [95]. In another study of adenocarcinoma type non-small-cell lung cancer (NSCLC) metabolic profiling of tissue samples revealed increased levels of uric acid [94]. Kynurenine (Kyn) and xanthine are metabolites also found to be related to oxidative stress occurrence in complex networks of cancer metabolism. Kyn is the first product in the pathway of Trp degradation and has several important biological processes. In addition, it also exhibits pro-oxidant effects. For example, aerobic irradiation of Kyn produces superoxide radical that leads to cytochrome C reduction while increased levels of Kyn have been shown to cause cell death through ROS pathway in natural killer (NK) cells [98–100]. Xanthine is an intermediate in the purine degradation process whose production might be accompanied by generation of H_2O_2 . This process is mediated by xanthine oxidase form of the xanthine oxidoreductase (XOR) [101]. Both, kynurenine and xanthine have been found to be specifically upregulated in different cancers (Table 1). A complete list of selected metabolites that were shown to be in any case connected to metabolic perturbations related to oxidative stress are considered in Table 1.

9. Conclusions

In this short review we have tried to put emphasis on the importance of pathophysiology of oxidative stress in cancer development in relation to metabolic adaptation of particular cancer cells and adjacent non-malignant cells with respect to the main stages of carcinogenesis according to the recent data obtained in this challenging field of cancer metabolomics. Our focus was primarily on biomarkers of oxidative stress. Comprehensive evidence gathered here, reveals a crucial role of oxidative stress in cancer development and progression. Yet, our understanding of the redox-related perturbations in cancer metabolism is still at the beginning, needing much more work before its exploitation in therapies and diagnosis. According to the collected data, we have prepared global biological network displaying complex interactions of altered metabolites (colored red) summed up in our review that were reported to be connected with the redox signalling in cancer and also with other specific pathway-associated metabolites (Fig. 1). Additionally, in Fig. 2, we have shown schematic representation of significantly altered metabolic pathways associated with the altered redox signalling in cancer, addressed in this paper.

In conclusion, we can claim that adaptation to oxidative stress is one of the main driving forces of cancer development, while thus generated lipid peroxidation might eventually help defending adjacent non-malignant cells from cancer invasion. Putting together parts of cancer puzzle is one of the main challenges in modern medicine and research. With the help of metabolomics approach many novel findings are now being revealed, giving a great promise for future scientific breakthroughs, thus supporting modern concepts of integrative medicine focused on pathophysiology of oxidative stress in cancer [107–111].

Conflicts of interest

None.

Dedication

On behalf of friends, pupils and followers of Hermann Esterbauer, the authors dedicate this paper to the memory on “Hermann of the Aldehydes” who passed away from cancer twenty years ago.

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